



SEP 20 2007

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Affidavit 1.132

I have worked as a clinical embryologist for fourteen years in the United States. I have been licensed practitioner of microsurgical fertilization (ICSI) and embryo biopsy in the UK. I have worked at Reprogenetics for eleven months and have been working to isolate disomic stem cells derived from trisomic embryos for 3 months. I am a relative novice in stem cell biology. I have read and examined Santiago Munne's patent application 20050019907 and the methods outlined in his publication. I have followed the protocols and methods outlined in his publication with some adaptations that minimize the use of animal components in the culture system. This work is preliminary and full characterization for pluripotency is not yet complete. A current cell line, derived from an embryo trisomic for chromosomes 16 and 18 and monosomy 22, from which disomic cells have been isolated, was cultured and characterized according to the following protocol.

Human embryos classified as chromosomally abnormal after Preimplantation Genetic Diagnosis (PGD) and not selected for the establishment of pregnancy were donated according to IRB approved consent (WIRB protocol 20041976) and signed by each patient. In most cases a single cell was biopsied from day 3 embryo and the nucleus analyzed for chromosome aneuploidy. When a nucleus was not observed in the first cell, a second was biopsied. All cells were analyzed for chromosomes X, Y, 13, 15, 18, 21, and 22 using FISH protocols described by Santiago Munne in the literature. Based on these results, individual embryos were selected for the study. Trisomic and monosomic embryos were continued in a culture of complex media (Global medium, IVF Online) until day 6 of development or blastocyst stage. Blastocysts with a visible inner cell mass (ICM) were selected for further culture. In this case, trophectoderm biopsy was not performed for the purpose of minimizing manipulations to maximize ongoing viability. Selected blastocysts were treated with pronase or acidified tyrodes solution (IVF Online) to dissolve the zona pellucida. Zona free intact blastocysts were plated onto human foreskin fibroblasts (HFF) (ATCC-CRL-1634) previously treated with mitotic inhibitor Mitomycin-C (Sigma-Aldrich). Media used to maintain HFF feeder cells was composed of KO-DMEM 80% (Invitrogen), certified fetal bovine serum 20% (Invitrogen) L-Glutamine 2mM (Invitrogen), NEAA X1 (Invitrogen), 2-β mercaptoethanol 0.1mM (Invitrogen), Pen/Strep x1 (Invitrogen).

Blastocysts were plated on HFF feeder cell monolayers and cultured at 37°C with 5% CO₂ and observed for attachment and primary outgrowth within 48hrs. Media was then changed every 24hrs and cultured for approximately 12 days until colony formation. Putative human embryonic stem cell (hESC) colonies were maintained in the following equilibrated xeno free chemically defined medium: DMEM F-12 (Invitrogen), 1x B-27 (stock:50) (Invitrogen), 1xN2 (stock:100) (Invitrogen), L-Glutamine 200mM (2mM final